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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
RAO, MANJUNATH N	
ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 05/15/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/003,759	WICHER ET AL.	
	Examiner	Art Unit	
	Manjunath N. Rao, Ph.D.	1652	

-- The MAILING DATE of this communicati n appears on the c ver sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *24 February 2003* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-22 and 30* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-22 and 30* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

DETAILED ACTION

Claims 1-22 and 30 are still at issue and are present for examination.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-22 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claims 1-22 all recite the phrase either "improved thermostable cellulase activity". The metes and bounds of the word "improved" is not clear to the Examiner. A perusal of the specification did not provide the Examiner, a specific definition for the above word in the context of the above claims thus rendering the claims indefinite.

Claims 1-22 and 30 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claims 1-22 and 30 recite the phrase "corresponding to the position one to about 40 in the corresponding SEQ ID NO:2" the metes and bounds of the phrase "corresponding to" is highly unclear to the Examiner. While it is clear to the Examiner that applicants have made an enzyme with improved activity in which the first 40 amino acid residues in SEQ ID NO:2 has been deleted, it is unclear to he Examiner as to what they mean by the phrase "corresponding to the position one to about 40 in the corresponding SEQ ID NO:2".

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-13, 15-22, 30 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a DNA sequence with SEQ ID NO:3 encoding a polypeptide with SEQ ID NO:2 having thermostable cellulase activity or DNA comprising SEQ ID NO:3 nucleotides 112-783 of SEQ ID NO:3 encoding a polypeptide with SEQ ID NO:2 in which the first 40 amino acids are deleted and having a thermostable cellulase activity that is at least two times greater than the specific activity of the polypeptide with SEQ ID NO:2, does not reasonably provide enablement for any variant DNA sequence that encodes a polypeptide whose amino acid sequence is 85% identical to the amino acid sequence of SEQ ID NO:2 or encoding any polypeptide that is truncated such that one or more amino acid residues corresponding to position one to about position 40 in SEQ ID NO:2 are deleted from any source including vectors, host cells comprising such DNA and method of making such truncated polypeptides. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

Factors to be considered in determining whether undue experimentation is required are summarized in In re Wands (858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir. 1988)) as follows: (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claim(s).

Claims 1-13, 15-22, 30 are so broad as to encompass any DNA, which encodes a variant glycosylhydrolase, polypeptide whose amino acid sequence is 85% identical to the amino acid sequence of SEQ ID NO:2 and wherein said polynucleotide is truncated such that one or more amino acid residues corresponding to position one to about position 40 in SEQ ID NO:2 are deleted. The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of DNA sequences that are broadly encompassed by the claims.

The applicants propose to use the above polynucleotides for the process of producing recombinant protein with improved characteristics. Furthermore, while applicants have shown that deletion of all the forty N-terminal amino acids in SEQ ID NO:2 results in a polypeptide with improved characteristics compared with the full length SEQ ID NO:2, they have not shown that deletion of just any one, two or three amino acids in the N-terminal region comprising the first 40 amino acids, results in the same effects. Therefore changing the nucleotide sequences as proposed by the applicants (i.e., a polynucleotide encoding a polypeptide that is 85% identical to SEQ ID NO:2 and wherein said polynucleotide is truncated such that one or more amino acid residues corresponding to position one to about position 40 in SEQ ID NO:2 are deleted) may not lead to desired function of the polynucleotides. It would require undue experimentation of the skilled artisan to make the claimed polynucleotides. The specification is limited to teaching the making of the polynucleotide which encodes a polypeptide in which all the first 40 N-terminal amino acids are deleted but provides no guidance with regard to making and using of polynucleotides encoding a polypeptide with improved characteristics in which only one, two or three amino acids from among the first 40 amino acids are deleted. In view of the great breadth

of the claim, amount of experimentation required to make the claimed polynucleotides encoding polypeptide with improved characteristics, the lack of guidance, working examples, and unpredictability of the art in predicting function from a polypeptide primary structure (e.g., see Ngo et al. in The Protein Folding Problem and Tertiary Structure Prediction, 1994, Merz et al. (ed.), Birkhauser, Boston, MA, pp. 433 and 492-495), the claimed invention would require undue experimentation. As such, the specification fails to teach one of ordinary skill how to make and use the full scope of the polynucleotides encompassed by the claims. This is because applicants have not provided any guidance to those skilled in the art regarding selecting specific amino acids in the N-terminal region for deletion. It would be a undue burden to those skilled in the art to determine which specific amino acid/s need to be deleted in a large group of polynucleotides encoding a polypeptide that is 85% identical to SEQ ID NO:2 and has improved characteristics. However, in this case the disclosure is limited to a single nucleotide encoding the polypeptide in which all the first 40 N-terminal amino acids are deleted.

While recombinant and mutagenesis techniques are known, it is not routine in the art to screen for multiple substitutions or modifications of nucleotides, as encompassed by the instant claims, and the base changes within a nucleic acid's sequence that can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited and the result of such modifications is unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given DNA to diminish with each further and additional modification, e.g. multiple substitutions.

The specification does not support the broad scope of the claims which encompass all modifications and fragments of any DNA encoding a protein having thermostable cellulase

activity because the specification does not establish: (A) that deleting even one single amino acid in the N-terminal region results in the DNA encoding a polypeptide with improved activity; (B) the general tolerance of the N-terminal amino acids (the first 40 amino acids) thermostable cellulase DNA sequence to modification and extent of such tolerance; (C) a rational and predictable scheme for modifying any thermostable cellulase nucleotide with an expectation of obtaining the desired biological function and utility; and (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including any variant DNA sequence that encodes a polypeptide whose amino acid sequence is 85% identical to the amino acid sequence of SEQ ID NO:2 wherein said polynucleotide is truncated such that one or more amino acid residues corresponding to position one to about position 40 in SEQ ID NO:2 are deleted. The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of DNAs having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re Wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

In response to the previous Office action, applicants have traversed the above rejection arguing that amended claims recite the structural and functional attributes that characterize the genus of the variants claimed. Applicants argue that embodiments recited sets forth a finite

number of possibilities that must be tested and therefor are not overly broad and considerable direction is provided. Applicants argue that the specification teaches region of DNA which may be modified without effecting enzymatic activity, (page 28-30 of the specification) and that the specification teaches that removal of one or more amino acids that are not part of the catalytic domain. Examiner respectfully disagrees. The specification at the above pages does not provide any guidance as to how one of ordinary skill in the art can arrive at a polynucleotide encoding a polypeptide that is 85% identical SEQ ID NO:2 and in addition have truncation in the N-terminal region. In the above pages the specification simply teaches expression of three specific variants and the testing of their activities. The specification does not explicitly teach that changes made in specific blocks of nucleotides encoding the polypeptide with SEQ ID NO:2 leads to improvement in the activity of the encoded polypeptide or the tolerance of the changes that would be acceptable.

Next applicants argue that using the teachings of the specification, one skilled in the art would be capable of making additions, deletions or substitutions and also test variants using the enzymatic assay. Applicants also argue that even though some experimentation is needed, courts have held that "enablement is not precluded by the necessity of some experimentation such as routine screening".

Examiner respectfully disagrees with such an argument as being persuasive to overcome the above rejection because while methods to produce variants of a known sequence, such as site-specific mutagenesis, random mutagenesis, etc. are well known to the skilled artisan, producing variants as claimed by applicants (i.e., a polynucleotide encoding a polypeptide with *improved activity* that is 85% identical SEQ ID NO:2 and in addition have truncation in the N-

terminal region.) requires that one of ordinary skill in the art know or be provided with guidance for the selection of specific nucleotide/s in said polynucleotide that can be changed as well as which of the infinite number of derived variants have the claimed property. Without such guidance one of ordinary skill would be reduced to the necessity of producing and testing all of the virtually infinite possibilities. Contrary to applicant's argument, this would clearly constitute undue experimentation. While enablement is not precluded by the necessity for routine screening, if a large amount of screening is required, the specification must provide a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. Such guidance has not been provided in the instant specification. As previously stated the specification does not establish: (A) regions of the protein structure which may be modified without effecting activity and thermostability; (B) the general tolerance of endoglucanases to modification and extent of such tolerance; (C) a rational and predictable scheme for modifying any amino acid residue with an expectation of obtaining the desired biological function; and (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful. Therefore the above rejection is maintained.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-22 and 30 are rejected under 35 U.S.C. 103(a) as obvious over Halldorsdottir et al. (Appl. Microbiol. Biotechnol., 1998, Vol. 49:277-284, and the enclosed sequence alignment), and Ohmiya et al. (J. Bacteriol., 1991, Vol. 173(2):636-641). This rejection is based upon the public availability of printed publications. Claims 1-22 and 30 of the instant application are drawn to a variant polynucleotide sequence encoding a polypeptide having improved thermostable cellulase activity and at least 85% identity to SEQ ID NO:2 wherein said polynucleotide is truncated such that one or more residues from position one to position 40 are deleted, or wherein 52-82 nucleotides from the 5' end are deleted (as depicted in claim 7), or wherein 88-112 nucleotides from the 5' end are deleted (as depicted in claim 11), or polynucleotides comprising sequences for a fusion protein comprising the sequences of the thermostable cellulase vectors and host cells comprising the same (claims 19-20, 22, 30) and methods of producing the thermostable cellulase. Halldorsdottir et al. teach the cloning, sequencing and over expression of a *R..marinus* gene encoding a thermostable cellulase. The reference provides the polynucleotide sequence as a GenBank deposit (GenBank Accession No.RMU72637, 11 May 1999). The reference also teaches vectors and host cells and method of making the thermostable cellulase from culturing the host cells. However, the reference does not teach the variant polynucleotide sequences encoding a polypeptide having improved thermostable cellulase activity and at least 85% identity to SEQ ID NO:2 wherein said polynucleotide is truncated such that one or more residues from position one to position 40 are deleted

Ohmiya et al. teach that N-terminal truncation of the signal sequence in a bacterial endoglucanase (cellulase) resulted in higher cellulase activity (see entire document, especially

figure 1 and page 638) compared to the wild type enzyme. Similarly deletion of 15 amino acids in the N-terminal in addition to the full signal sequence resulted in even higher activity compared to the activities of the wild type enzyme and the first variant above. The reference also teaches that deletion of more amino acids in the N-terminal region does not continue to an increase activity. Thus those skilled in the art can readily infer that deletion of the signal sequence and up to about 15 amino acids in addition to the signal sequence amino acids at the N-terminal region in a cellulase (endoglucanase) results in a variant that has improved activity.

With the above two reference in hand and combining the teachings of the above two references, it would have been obvious to one of ordinary skill in the art at the time this application was filed to use the polynucleotide provided by Halldorsdottir et al. and make a variant polynucleotide in which the signal sequence and up to about 15 amino acids, i.e., the first 30-40 amino acids are deleted such that it encodes a polypeptide which would be reasonably expected to have improved thermostable cellulase activity. Ohmiya et al. teach that one of ordinary skill in the art would have been motivated to construct these deletions in order to make endoglucanase with improved cellulase activity. One or ordinary skill in the art would have a reasonable expectation of success since Halldorsdottir et al. teach the cloning, sequencing and over expression of a thermostable cellulase from *R.marinus* and Ohmiya et al. teach that deletion of N-terminal amino acids including the signal sequence can result in improved activity of another bacterial endoglucanase.

Therefore the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103(a).

In response to the previous Office action, applicants have traversed the above rejection, arguing that applicants invention pertains to the unexpected discovery that removal of the N-terminal hydrophobic region and/or linker moiety of glycosylhydrolases of family 12 yields to polypeptides of thermostable cellulases with superior catalytic and physical properties when compared to native full length polypeptide. While applicants simply claim a polypeptide with "improved thermostable activity", in their arguments they argue that their invention pertains to a variant polypeptide having a three-fold increase in the specific activity and more stability compared to wild type polypeptide. Applicants also argue that they demonstrated that the N-terminal end was detrimental to the cells and further removal of amino acids at the N-terminal end produced variants with superior properties, even though such characteristics are not claim limitations. Applicants continue to argue based on limitations not claimed in the claims and argue that such limitations are not taught by the reference. Examiner respectfully disagrees with such arguments and reiterates that such arguments does not overcome the above rejection because all the characteristics of the encoded polypeptide argued by the applicants are not claim limitations.

Applicants also argue that the Gilkes et al. reference does not apply. However, such arguments are moot as Examiner has withdrawn the above reference and rewritten the rejection based on a new reference. Applicants mainly appear to argue that their invention is based on unexpected results. Applicants also provide the Examiner with a Declaration to support unexpected results. Examiner respectfully disagrees with the applicants that they have observed unexpected results in view of the new reference provided by the Examiner. Based on the new reference Examiner takes the position that the fact that truncation of N-terminal amino acids in a glycosyl hydrolase such as a cellulase was well known in the art and that one would have expected a increased activity to result from such truncation. Therefore, the above invention would have been *prima facie* obvious to one of ordinary skill in the art.

Conclusion

None of the claims are allowable.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Manjunath N. Rao, Ph.D. whose telephone number is 703-306-5681. The examiner can normally be reached on 7.30 a.m. to 4.00 p.m. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura Achutamurthy can be reached on 703-308-3804. The fax phone numbers for the organization where this application or proceeding is assigned are 703-308-4242 for regular communications and 703-308-4242 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 703-306-0196.

MANJUNATH RAO
PATENT EXAMINER

Manjunath N. Rao
May 14, 2003